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(71) Applicant : **ELI LILLY AND COMPANY**  
**Lilly Corporate Center**  
**Indianapolis Indiana 46285 (US)**

(72) Inventor : **Alborn, William Ernest, Jr.**  
**1320 Thistlewood Court**  
**Carmel, Indiana 46032 (US)**

Inventor : **Hoskins, Jo Ann**  
**8229 Tern Court**  
**Indianapolis, Indiana 46256 (US)**  
Inventor : **Skatrud, Paul Luther**  
**2412 Lake Crossing**  
**Greenwood, Indiana 46143 (US)**  
Inventor : **Ünal, Serhat**  
**Yesilyurt sok. No: 7/8**  
**06690 Kavakalidere, Ankara (TR)**

(74) Representative : **Hudson, Christopher Mark et al**  
**Lilly Industries Limited**  
**European Patent Operations**  
**Erl Wood Manor**  
**Windlesham Surrey GU20 6PH (GB)**  
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(54) **Fem A gene of staphylococcus epidermidis, fem A protein, and vectors of microorganisms comprising the fem A gene.**

(57) The instant invention provides the *femA* gene of *Staphylococcus epidermidis* and all degenerate sequences thereof, the protein encoded by the *femA* gene (FemA), and vectors and microorganisms comprising genes encoding the FemA protein.

Clinical isolates of staphylococci (*Staphylococcus aureus* and *S. epidermidis*) which cause serious infections due to their intrinsic resistance to beta-lactamase-stable beta-lactam antibiotics (e.g., methicillin) carry the *mecA* gene. Song et al., *FEBS Lett.* **221**:167-171 (1987). This gene encodes a putative cell wall biosynthetic enzyme referred to as penicillin binding protein 2a (PBP2a). PBP2a, which binds beta-lactams only at concentrations well above therapeutic efficacy, apparently can functionally substitute for all the staphylococcal PBPs and permit growth when the host organism is threatened by beta-lactams. Hartman and Tomasz, *J. Bacteriol.* **158**:513-516 (1984). Wu et al., *Antimicrob. Agents Chemother.* **36**:533-539 (1992) and Ryffel et al., *Gene* **94**:137-138 (1990).

The *mecA* gene is not a normal part of the staphylococcal genome. The organism which donated *mecA* to the staphylococci remains unidentified. Despite the uniform presence of *mecA* in methicillin-resistant clinical isolates, these isolates vary considerably in their degree of resistance to methicillin. This variation in phenotypic expression within a population has been referred to as heterogenous expression. Matthews and Stewart, *FEMS Microbiol. Lett.* **22**:161-166 (1984). Typically, most cells exhibit low-level resistance to methicillin and only a minority of the population express high-level resistance, perhaps only one in  $10^8$  cells. Tomasz et al., *Antimicrob. Agents Chemother.* **35**:124-129 (1991). Although expression of methicillin resistance is dependent upon the presence of PBP2a, it appears to be somewhat independent of the amount of PBP2a, suggesting important roles for other factors. Chambers and Hackbarth, *Antimicrob. Agents Chemother.* **31**:1982-1988 (1987) and Murakami and Tomasz, *J. Bacteriol.* **171**:874-879 (1989).

Tn551 insertional mutagenesis of methicillin-resistant *S. aureus* revealed numerous sites which influence the level of methicillin resistance but are not linked to *mecA* and do not perturb the expression of PBP2a. Berger-Bächi et al., *Antimicrob. Agents Chemother.* **36**:1367-1373 (1992); Kornblum et al., *Eur. J. Clin. Microbiol.* **5**:714-718 (1986); Berger-Bächi et al., *Mol. Gen. Genet.* **219**:263-269 (1989) and Maidhof et al., *J. Bacteriol.* **173**:3507-3513 (1991). Those factors described thus far generally depress the MIC of beta-lactam resistant strains. Some of the genetic loci which demonstrate such an effect on methicillin resistance were designated factors essential for methicillin resistance (*fem*). Berger-Bächi et al., *Mol. Gen. Genet.* **219**:263-269 (1989). In contrast to *mecA*, the genes which encode influential factors are probably present in both resistant and susceptible strains of *S. aureus* and *S. epidermidis*. Information obtained from gene disruption studies of *femA* and *femB* in *S. aureus* indicated that in addition to enhanced sensitivity to methicillin, homogeneously methicillin-resistant *S. aureus* strains carrying such gene disruptions have a reduced glycine content in the peptidoglycan component of their cell walls (Maidhof et al., *J. Bacteriol.* **173**:3507-3513 (1991)) and exhibit reduced rates of cell wall turnover and autolysis (de Jonge et al., *J. Bacteriol.* **173**:1105-1110 (1991)).

Genetic factors, other than *mecA*, that influence the expression of methicillin resistance in *S. epidermidis* have, until now, not been described at the molecular level. The present invention provides DNA sequences encoding the FemA protein of *Staphylococcus epidermidis*, the FemA protein itself, and vectors and microorganisms comprising the *femA* gene of *S. epidermidis*.

The present invention provides DNA sequences encoding the FemA protein of *Staphylococcus epidermidis*, including the natural gene sequence designated *femA* (SEQ ID NO:1). Thus, included in the present invention is any DNA compound that comprises an isolated DNA sequence encoding SEQ ID NO:2. SEQ ID NO:2 is as follows:

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Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe  
1 5 10 15

5 Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr  
20 25 30

Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys  
35 40 45

10 Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro  
50 55 60

Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile  
65 70 75 80

15 Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser  
85 90 95

Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr  
100 105 110

20 Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala  
115 120 125

Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His  
130 135 140

25 Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His  
145 150 155 160

30 Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn  
165 170 175

Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly  
180 185 190

35 Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser  
195 200 205

Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp  
210 215 220

40 Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val  
225 230 235 240

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Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn  
 245 250 255  
 5 Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile  
 260 265 270  
 Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu  
 275 280 285  
 10 Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn  
 290 295 300  
 Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe  
 305 310 315 320  
 15 Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn  
 325 330 335  
 Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile  
 340 345 350  
 20 Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile  
 355 360 365  
 Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe  
 370 375 380  
 25 Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile  
 385 390 395 400  
 Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys  
 405 410 415  
 30 Leu Lys Lys

The natural *femA* sequence is encompassed by the present invention as a DNA compound which com-  
 35 prises the isolated DNA sequence which is SEQ ID NO:1. SEQ ID NO:1 is as follows:

ATG AAG ATG AAG TTT ACG AAT TTG ACA GCT AAA GAA TTT AGT GAC TTT 48  
 Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe  
 40 1 5 10 15  
 ACT GAT CGT ATG ACA TAT AGT CAT TTT ACA CAA ATG GAA GGT AAT TAC 96  
 Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr  
 20 25 30  
 45  
 50  
 55

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GAA TTA AAG GTT GCT GAA GGT ACC GAG TCA CAT TTA GTT GGA ATT AAA 144  
 Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys  
 35 40 45

5 AAT AAT GAT AAC GAA GTG ATT GCA GCT TGT TTA TTA ACA GCT GTT CCT 192  
 Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro  
 50 55 60

10 GTA ATG AAA ATA TTT AAA TAT TTT TAT TCC AAT CGC GGT CCA GTA ATA 240  
 Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile  
 65 70 75 80

15 GAT TAT AAT AAT AAA GAG CTT GTA CAT TTT TTC TTT AAT GAA TTG AGT 288  
 Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser  
 85 90 95

AAA TAT GTA AAA AAA TAT AAT TGT TTA TAT TTA AGA GTT GAC CCA TAC 336  
 Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr  
 100 105 110

20 CTT CCA TAT CAA TAT TTA AAT CAT GAG GGA GAA ATA ACT GGA AAT GCA 384  
 Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala  
 115 120 125

25 GGT CAT GAT TGG ATT TTT GAT GAA TTA GAG AGT TTA GGA TAT AAA CAC 432  
 Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His  
 130 135 140

GAA GGA TTC CAC AAA GGA TTT GAT CCT GTA TTA CAA ATC CGA TAT CAT 480  
 Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His  
 145 150 155 160

30 TCT GTT CTA AAT TTA GCA AAC AAA AGT GCT AAT GAT GTT TTA AAA AAC 528  
 Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn  
 165 170 175

35 ATG GAT GGT TTA AGA AAG CGT AAT ACT AAA AAA GTT AAG AAA AAT GGA 576  
 Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly  
 180 185 190

GTT AAA GTC CGC TTT TTA TCT GAA GAA GAG TTA CCT ATA TTT AGG TCA 624  
 Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser  
 195 200 205

40 TTT ATG GAG GAT ACC TCT GAA ACT AAA GAT TTT GCA GAT AGA GAA GAT 672  
 Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp  
 210 215 220

45 AGT TTT TAT TAC AAC AGA TTC AAA CAT TAT AAA GAC CGT GTT TTA GTA 720  
 Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val  
 225 230 235 240

50 CCA CTA GCC TAT ATT AAC TTT GAT GAG TAT ATA GAG GAA CTA AAT AAT 768  
 Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn  
 245 250 255

GAA AGA AAT GTG CTT AAT AAA GAT TAT AAT AAA GCT TTA AAA GAC ATT 816  
 Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile  
 260 265 270

5 GAG AAA CGT CCA GAG AAT AAA AAA GCA CAT AAC AAA AAG GAA AAT TTA 864  
 Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu  
 275 280 285

10 GAA CAA CAA CTC GAT GCA AAT CAG CAA AAA ATT AAT GAA GCT AAA AAC 912  
 Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn  
 290 295 300

15 TTA AAA CAA GAA CAT GGC AAT GAA TTA CCC ATC TCT GCT GGC TTC TTT 960  
 Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe  
 305 310 315 320

20 ATA ATT AAT CCG TTT GAA GTA GTT TAC TAC GCT GGT GGA ACT TCA AAT 1008  
 Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn  
 325 330 335

25 CGT TAT CGC CAT TTT GCA GGG AGC TAT GCG GTT CAA TGG AAG ATG ATT 1056  
 Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile  
 340 345 350

AAC TAT GCA ATT GAA CAT GGT ATT AAT CGG TAT AAT TTC TAT GGT ATT 1104  
 Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile  
 355 360 365

30 AGT GGT GAC TTT AGT GAA GAT GCT GAA GAT GCT GGC GTA GTT AAG TTT 1152  
 Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe  
 370 375 380

AAA AAG GGC TAT GAT GCC GAT GTT ATA GAA TAC GTT GGT GAC TTT ATT 1200  
 Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile  
 385 390 395 400

35 AAA CCT ATT AAT AAA CCA ATG TAT AAC ATT TAT AGA ACA CTT AAA AAA 1248  
 Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys  
 405 410 415

CTA AAG AAA 1257  
 Leu Lys Lys

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The present invention also includes the protein encoded by SEQ ID NO: 1 in purified form. Also included are recombinant DNA vectors, including expression vectors, that comprise DNA sequences encoding FemA.

The restriction site and function maps presented in the accompanying drawings are approximate representations of the recombinant DNA vectors discussed herein. The restriction site information is not exhaustive; therefore, there may be more restriction sites of a given type on the vector than actually shown on the map.

45 Figure 1 - A restriction site and function map of plasmid pPSJ180.

Figure 2 - A restriction site and function map of plasmid pET-11A.

The instant invention provides the *femA* gene of *Staphylococcus epidermidis* and all degenerate sequences thereof, the protein encoded by the *femA* gene (FemA), and vectors and microorganisms comprising genes encoding the FemA protein. In the practice of the invention as exemplified herein, the FemA protein comprises the amino acid sequence, which is SEQ ID NO 2:

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	Met	Lys	Met	Lys	Phe	Thr	Asn	Leu	Thr	Ala	Lys	Glu	Phe	Ser	Asp	Phe
	1				5					10					15	
5	Thr	Asp	Arg	Met	Thr	Tyr	Ser	His	Phe	Thr	Gln	Met	Glu	Gly	Asn	Tyr
				20					25					30		
	Glu	Leu	Lys	Val	Ala	Glu	Gly	Thr	Glu	Ser	His	Leu	Val	Gly	Ile	Lys
			35					40					45			
10	Asn	Asn	Asp	Asn	Glu	Val	Ile	Ala	Ala	Cys	Leu	Leu	Thr	Ala	Val	Pro
		50					55					60				
	Val	Met	Lys	Ile	Phe	Lys	Tyr	Phe	Tyr	Ser	Asn	Arg	Gly	Pro	Val	Ile
	65					70					75					80
15	Asp	Tyr	Asn	Asn	Lys	Glu	Leu	Val	His	Phe	Phe	Phe	Asn	Glu	Leu	Ser
					85					90					95	
	Lys	Tyr	Val	Lys	Lys	Tyr	Asn	Cys	Leu	Tyr	Leu	Arg	Val	Asp	Pro	Tyr
				100					105					110		
20	Leu	Pro	Tyr	Gln	Tyr	Leu	Asn	His	Glu	Gly	Glu	Ile	Thr	Gly	Asn	Ala
			115					120					125			
	Gly	His	Asp	Trp	Ile	Phe	Asp	Glu	Leu	Glu	Ser	Leu	Gly	Tyr	Lys	His
25		130					135					140				
	Glu	Gly	Phe	His	Lys	Gly	Phe	Asp	Pro	Val	Leu	Gln	Ile	Arg	Tyr	His
	145					150					155					160

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Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn  
 165 170 175  
 5 Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly  
 180 185 190  
 Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser  
 195 200 205  
 10 Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp  
 210 215 220  
 Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val  
 225 230 235 240  
 15 Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn  
 245 250 255  
 Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile  
 260 265 270  
 20 Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu  
 275 280 285  
 Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn  
 290 295 300  
 25 Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe  
 305 310 315 320  
 Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn  
 325 330 335  
 30 Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile  
 340 345 350  
 35 Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile  
 355 360 365  
 Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe  
 370 375 380  
 40 Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile  
 385 390 395 400  
 Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys  
 405 410 415  
 45 Leu Lys Lys

The present invention also provides the natural *femA* gene found in *Staphylococcus epidermidis*, embod-  
 ied in SEQ ID NO: 1:

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ATG AAG ATG AAG TTT ACG AAT TTG ACA GCT AAA GAA TTT AGT GAC TTT 48  
 Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe  
 1 5 10 15  
 ACT GAT CGT ATG ACA TAT AGT CAT TTT ACA CAA ATG GAA GGT AAT TAC 96  
 Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr  
 20 25 30  
 GAA TTA AAG GTT GCT GAA GGT ACC GAG TCA CAT TTA GTT GGA ATT AAA 144  
 Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys  
 35 40 45  
 AAT AAT GAT AAC GAA GTG ATT GCA GCT TGT TTA TTA ACA GCT GTT CCT 192  
 Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro  
 50 55 60  
 GTA ATG AAA ATA TTT AAA TAT TTT TAT TCC AAT CGC GGT CCA GTA ATA 240  
 Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile  
 65 70 75 80  
 GAT TAT AAT AAT AAA GAG CTT GTA CAT TTT TTC TTT AAT GAA TTG AGT 288  
 Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser  
 85 90 95  
 AAA TAT GTA AAA AAA TAT AAT TGT TTA TAT TTA AGA GTT GAC CCA TAC 336  
 Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr  
 100 105 110  
 CTT CCA TAT CAA TAT TTA AAT CAT GAG GGA GAA ATA ACT GGA AAT GCA 384  
 Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala  
 115 120 125  
 GGT CAT GAT TGG ATT TTT GAT GAA TTA GAG AGT TTA GGA TAT AAA CAC 432  
 Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His  
 130 135 140  
 GAA GGA TTC CAC AAA GGA TTT GAT CCT GTA TTA CAA ATC CGA TAT CAT 480  
 Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His  
 145 150 155 160  
 TCT GTT CTA AAT TTA GCA AAC AAA AGT GCT AAT GAT GTT TTA AAA AAC 528  
 Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn  
 165 170 175  
 ATG GAT GGT TTA AGA AAG CGT AAT ACT AAA AAA GTT AAG AAA AAT GGA 576  
 Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly  
 180 185 190

5 GTT AAA GTC CGC TTT TTA TCT GAA GAA GAG TTA CCT ATA TTT AGG TCA 624  
 Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser  
 195 200 205

TTT ATG GAG GAT ACC TCT GAA ACT AAA GAT TTT GCA GAT ACA GAA GAT 672  
 Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp  
 210 215 220

10 AGT TTT TAT TAC AAC AGA TTC AAA CAT TAT AAA GAC CGT GTT TTA GTA 720  
 Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val  
 225 230 235 240

15 CCA CTA GCC TAT ATT AAC TTT GAT GAG TAT ATA GAG GAA CTA AAT AAT 768  
 Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn  
 245 250 255

GAA AGA AAT GTG CTT AAT AAA GAT TAT AAT AAA GCT TTA AAA GAC ATT 816  
 Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile  
 260 265 270

20 GAG AAA CGT CCA GAG AAT AAA AAA GCA CAT AAC AAA AAG GAA AAT TTA 864  
 Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu  
 275 280 285

25 GAA CAA CAA CTC GAT GCA AAT CAG CAA AAA ATT AAT GAA GCT AAA AAC 912  
 Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn  
 290 295 300

30 TTA AAA CAA GAA CAT GGC AAT GAA TTA CCC ATC TCT GCT GGC TTC TTT 960  
 Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe  
 305 310 315 320

ATA ATT AAT CCG TTT GAA GTA GTT TAC TAC GCT GGT GGA ACT TCA AAT 1008  
 Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn  
 325 330 335

35 CGT TAT CGC CAT TTT GCA GGG AGC TAT GCG GTT CAA TGG AAG ATG ATT 1056  
 Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile  
 340 345 350

AAC TAT GCA ATT GAA CAT GGT ATT AAT CGG TAT AAT TTC TAT GGT ATT 1104  
 Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile  
 355 360 365

40 AGT GGT GAC TTT AGT GAA GAT GCT GAA GAT GCT GGC GTA GTT AAG TTT 1152  
 Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe  
 370 375 380

45 AAA AAG GGC TAT GAT GCC GAT GTT ATA GAA TAC GTT GGT GAC TTT ATT 1200  
 Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile  
 385 390 395 400

50 AAA CCT ATT AAT AAA CCA ATG TAT AAC ATT TAT AGA ACA CTT AAA AAA 1248  
 Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys  
 405 410 415

55 CTA AAG AAA 1257  
 Leu Lys Lys

The synthesis of the FemA protein of the present invention may proceed by solid phase peptide synthesis or by recombinant methods. Both methods are described in U.S. Patent No. 4,617,149, the entire teaching of which is herein incorporated by reference. Recombinant methods are preferred if a high yield is desired. The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area such as Dugas, H. and Penney, C., *Bioorganic Chemistry* (1981), Springer-Verlag, New York, pgs. 54-92.

Synthesis of the FemA protein can be achieved by recombinant DNA technology. Synthetic genes, the *in vitro* or *in vivo* transcription and translation of which will result in the production of the FemA protein may be constructed by techniques well known in the art. Owing to the natural degeneracy of the genetic code, the skilled artisan will recognize that a sizable yet definite number of DNA sequences may be constructed which encode the FemA protein. All such genes are provided by the present invention. A preferred gene encoding the FemA protein is the natural *femA* gene of *Staphylococcus epidermidis*, which is SEQ ID NO: 1. This preferred *femA* gene is available on an ~ 3.7 kb *EcoRI* restriction fragment of plasmid pPSJ180, publicly available and on deposit in *Escherichia coli* DH5 $\alpha$  at the National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, Illinois 61604-39999, under accession number NRRL B-21024 (date of deposit: December 8, 1992). A restriction site and function map of pPSJ180 is provided in Figure 1 of the drawings.

The *femA* gene may be created by synthetic methodology. Such methodology of synthetic-gene construction is well known in the art. See Brown et al. (1979) *Methods in Enzymology*, Academic Press, N.Y., 68:109-151. The *femA* DNA sequence may be generated using a conventional DNA synthesizing apparatus such as the Applied Biosystems Model 380A or 380B DNA synthesizers (commercially available from Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404).

To effect the translation of the FemA protein, one inserts the engineered synthetic DNA sequence in any of a large number of appropriate recombinant DNA expression vectors through the use of appropriate restriction endonucleases and DNA ligase. The synthetic *femA* gene should be designed to possess restriction endonuclease cleavage sites at either end of the transcript to facilitate isolation from and integration into these amplification and expression plasmids. The particular endonucleases employed will be dictated by the restriction endonuclease cleavage pattern of the parent expression vector to be employed. The choice of restriction sites are chosen so as to properly orient the FemA coding sequence with control sequences to achieve proper in-frame reading and expression of the FemA molecule. The FemA coding sequence must be positioned so as to be in proper reading frame with the promoter and ribosome binding site of the expression vector, both of which are functional in the host cell in which the FemA protein is to be expressed. The FemA protein may be expressed in any number of well-known eucaryotic or procaryotic hosts using known promoters and vectors. Some of the potential hosts, in addition to *E. coli*, include the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris*, *Bacillus*, and cells infected with baculovirus.

To achieve efficient transcription of the synthetic gene, said gene must be operably associated with a promoter operator region. In one practice of the invention, the promoter-operator region of the synthetic gene encoding SEQ ID NO: 2 is placed in the same sequential orientation with respect to the ATG start codon of the synthetic gene as the promoter-operator occupies with respect to the ATG-start codon of the gene from which it was derived. Synthetic or modified promoter operator regions have been created and are well known in the art. When employing such synthetic or modified promoter-operator regions they should be oriented with respect to the ATG-start codon of the *femA* gene as directed by their creators. In one practice of the invention as exemplified herein, where the host cell is an *E. Coli* host cell, said promoter-operator region is the phage T7 promoter-operator region.

A variety of expression vectors useful for transforming procaryotic cells are well known in the art. A preferred vector for expression in an *E. coli* host cell is derived from *E. coli* plasmid pET-11A, which comprises the phage T7 promoter. A restriction site and function map of pET-11A appears in Figure 2 of the accompanying drawings. Plasmid pET-11A is publicly available from Novagen, Inc. (565 Science Drive, Madison, WI 53711) under catalog #69436-1. The preferred host strain is *E. coli* BL21(DE3), also available from Novagen under catalog #69387-1.

The techniques of transforming cells with the aforementioned vectors are well known in the art and may be found in such general references as Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1988), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY or Ausubel et al., *Current Protocols in Molecular Biology* (1989), John Wiley & Sons, New York, NY and supplements. The techniques involved in the transformation of *E. coli* cells used in the preferred practice of the invention as exemplified herein are well known in the art. The precise conditions under which the transformed *E. coli* cells are cultured is dependent on the nature of the *E. coli* host cell line and the expression or cloning vectors employed. For example, vectors which incorporate thermoinducible promoter-operator regions, such as the cl857 thermoinducible lambda-phage promoter-operator region, require a temperature shift from about 30 to about 40°C. in the culture conditions so as to induce

protein synthesis.

In a preferred embodiment of the invention *E. coli* K12 BL21 (DE3) cells were employed as host cells but numerous other cell lines are available. The transformed host cells are then plated on appropriate media under the selective pressure of the antibiotic corresponding to the resistance gene present on the expression plasmid. The cultures are then incubated for a time and temperature appropriate to the host cell line employed. Specifically, with *E. coli* K12 BL21 (DE3) cells, the *femA* gene is placed under the control of a promoter transcribed specifically by the T7 RNA polymerase. Induction of transcription of the *femA* gene is accomplished by the addition of isopropylthiogalactoside (IPTG) to the growth medium, which induces expression of the T7 RNA polymerase gene under the control of the *lacUV5* promoter. The T7 RNA polymerase is then available to transcribe the *femA* gene.

General techniques of protein purification are well-known to those of ordinary skill in the art. See Creighton, T.E., *Protein Structure: A Practical Approach* (1989), IRL Press, Oxford, England and Bollag, D.M. and Edelstein, S.J., *Protein Methods* (1991), Wiley-Liss, New York, NY. Proteins which are expressed in high-level bacterial expression systems characteristically aggregate in granules or inclusion bodies which contain high levels of the overexpressed protein. Kreuger et al. (1990) in *Protein Folding*, Gierasch and King, eds., pgs 136-142, American Association for the Advancement of Science Publication No. 89-18S, Washington, D.C. and Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1988), pp. 17.37-17.41, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. The FemA protein sometimes aggregates into inclusion bodies when expressed under the control of phage T7 promoter. Such protein aggregates must be solubilized to provide further purification and isolation of the desired protein product. A variety of techniques using strongly denaturing solutions such as guanidinium-HCl and/or weakly denaturing solutions such as dithiothreitol (DTT) are used to solubilize the proteins.

Recombinantly produced proteins may be purified by a variety of techniques well known in the art such as ion exchange chromatography, size exclusion chromatography, electrophoresis, differential centrifugation, reversed phase high performance liquid chromatography, immunoaffinity chromatography, and the like. Protocols for use of these individual techniques or combinations thereof are well known in the art. Gradual removal of the denaturing agents (often by dialysis) in a refolding solution allows the denatured protein to assume its native conformation. The particular conditions for denaturation and refolding are determined by the particular protein expression system and/or protein in question. The S-sulfonates of the peptide molecules are converted to the disulfide paired, folded FemA molecules using a combination of high pH and added thiol in substantial accordance with the teaching of Frank, B.H. et al., (1981) in *Peptides. Synthesis, Structure and Function. Proceedings of the Seventh American Peptide Symposium* (Rich, D.H. and Gross, E., eds.) pp. 729-738, Pierce Chemical Co., Rockford, IL.

The *femA* gene may be used in gene disruption studies in *Staphylococcus epidermidis*. Although it is believed that the FemA protein is involved in the formation of a pentaglycine bridge in the cell wall of the bacterium, gene disruption will allow one to ascertain the precise effect of the loss of the *femA* gene. Gene disruption experiments in *Staphylococcus aureus* have revealed that a loss of *femA* results in an ~40% reduction in cell wall glycine content. A similar result might be anticipated for *S. epidermidis*. Once determined, this information can be used to generate an assay for agents which inhibit the FemA protein, and are therefore useful in combination with antibiotics to treat methicillin-resistant bacteria.

The FemA protein of SEQ ID NO: 2 may be produced by recombinant methods. Recombinant methods are preferred if a high yield is desired. The present invention thus comprises a method for constructing a recombinant host cell capable of expressing SEQ ID NO: 2, said method comprising transforming a host cell with a recombinant DNA vector that comprises an isolated DNA sequence of Claim 1. The present invention also comprises a method for expressing SEQ ID NO: 2 in a recombinant host cell; said method comprising culturing said transformed host cell of Claim 5 under conditions suitable for gene expression.

The following Examples are provided to further illustrate and exemplify, but not limit the scope of, the invention.

## Example 1

### Source of the *Staphylococcus epidermidis* *femA* gene

#### Isolation of Plasmid pPSJ180

Alyophil of *E. coli* K12 DH5 $\alpha$ /pPSJ180 can be obtained from the Northern Regional Research Laboratories (NRRL), Peoria, Illinois 61604, under the accession number NRRL B-21024 (date of deposit: December 8, 1992). The pPSJ180 plasmid may be isolated from *E. coli* K12 DH5 $\alpha$ /pPSJ180 using techniques well-known

to those skilled in the art. See Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1988), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY or Ausubel et al., *Current Protocols in Molecular Biology* (1989), John Wiley & Sons, New York, NY and supplements.

#### 5 Isolation of the *Staphylococcus epidermidis* *femA* gene via the polymerase chain reaction

Isolated plasmid pPSJ180 is used as the template for the polymerase chain reaction at a concentration of 10 ng/reaction. Vent<sub>R</sub><sup>TM</sup> DNA polymerase (2 units/μl, Catalog #254, New England Biolabs, 32 Tozer Road, Beverly, MA 01915-9965) is used with standard Vent<sub>R</sub><sup>TM</sup> DNA polymerase buffer (1X= 10 mM KCl, 20 mM Tris-HCl (pH 8.8 at 25°C), 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 0.1% Triton X-100). The PCR primers used are AGATA-TAAAGATCTAGATGGGAGTTATGAA (SEQ ID NO: 3) and ATTCATAATTAGATGGATCCCTTCTTAAAATC (SEQ ID NO: 4). The reaction is carried out by 3 cycles of 94°C for 15 seconds, 40°C for 15 seconds and 72°C for 1 minute followed by 20 cycles of 94°C for 15 seconds, 55°C for 15 seconds and 72°C for 1 minute.

The reaction is transferred to a Centricon 100 microconcentrator (Amicon, Inc., 72 Cherry Hill, Beverly, MA 01915) and washed with 1 ml of water. The microconcentrator is then subjected to centrifugation at 3000 rpm in a microcentrifuge for 30 minutes. The reaction is then diluted to 200 μl (from ~50 μl) with 1X *Xba*I restriction enzyme buffer. To this is added 50 units of *Xba*I and 50 units of *Bam*HI. The DNA is then digested for 90 minutes at 37°C. The DNA is phenol extracted and ethanol precipitated.

#### 20 Example 2

##### Construction of an Expression Plasmid Containing the *femA* Gene

The DNA created in Example 1 is then ligated to the 5.6 kb *Xba*I-*Bam*HI fragment of pET-11A (available from Novagen, Inc. (565 Science Drive, Madison, WI 53711) under catalog #69436-1. This plasmid is then transformed into *E. coli* BL21 (DE3) (also available from Novagen, Inc. under catalog #69387-1) using techniques well known to those of ordinary skill in the art.

#### 30 Example 3

##### Expression of the FemA Protein

*E. coli* BL21 (DE3) transformed with the *femA* expression plasmid are grown overnight in TY broth (per liter 10 g tryptone, 5 g yeast extract and 5 g NaCl) and 100 μg/ml ampicillin. The cells are then diluted 1/50 into TY broth + ampicillin and grown at 37°C for 60 minutes. Expression is induced by adding isopropylthiogalactoside (IPTG) to 0.4 mM. Samples are taken at 0 and 6 hours and run on a % SDS-polyacrylamide gel using techniques described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1988), pp. 18.47-18.59, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. An induced protein band is visible by staining with Coomassie Blue at the predicted size of 49,000 daltons.

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SEQUENCE LISTING

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(1) GENERAL INFORMATION:

- (i) APPLICANT: ELI LILLY AND COMPANY  
(B) STREET: Lilly Corporate Center  
10 (C) CITY: Indianapolis  
(D) STATE: Indiana  
(E) COUNTRY: United States of America  
(F) ZIP: 46285
- 15 (ii) TITLE OF INVENTION: FEMA GENE OF STAPHYLOCOCCUS  
EPIDERMIDIS, FEMA PROTEIN, AND VECTORS AND  
MICROORGANISMS COMPRISING THE FEMA GENE
- (iii) NUMBER OF SEQUENCES: 4
- 20 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: C. M. Hudson  
(B) STREET: Erl Wood Manor  
(C) CITY: Windlesham  
25 (D) STATE: Surrey  
(E) COUNTRY: United Kingdom  
(F) ZIP: GU20 6PH
- (v) COMPUTER READABLE FORM:
- 30 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: Macintosh  
(C) OPERATING SYSTEM: Macintosh 7.0  
(D) SOFTWARE: Microsoft Word 5.1

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1257 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1257

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG	AAG	ATG	AAG	TTT	ACG	AAT	TTG	ACA	GCT	AAA	GAA	TTT	AGT	GAC	TTT	48
Met	Lys	Met	Lys	Phe	Thr	Asn	Leu	Thr	Ala	Lys	Glu	Phe	Ser	Asp	Phe	
1				5					10					15		

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ACT GAT CGT ATG ACA TAT AGT CAT TTT ACA CAA ATG GAA GGT AAT TAC 96  
 Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr  
 20 25 30

5 GAA TTA AAG GTT GCT GAA GGT ACC GAG TCA CAT TTA GTT GGA ATT AAA 144  
 Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys  
 35 40 45

10 AAT AAT GAT AAC GAA GTG ATT GCA GCT TGT TTA TTA ACA GCT GTT CCT 192  
 Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro  
 50 55 60

15 GTA ATG AAA ATA TTT AAA TAT TTT TAT TCC AAT CGC GGT CCA GTA ATA 240  
 Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile  
 65 70 75 80

GAT TAT AAT AAT AAA GAG CTT GTA CAT TTT TTC TTT AAT GAA TTG AGT 288  
 Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser  
 85 90 95

20 AAA TAT GTA AAA AAA TAT AAT TGT TTA TAT TTA AGA GTT GAC CCA TAC 336  
 Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr  
 100 105 110

25 CTT CCA TAT CAA TAT TTA AAT CAT GAG GGA GAA ATA ACT GGA AAT GCA 384  
 Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala  
 115 120 125

GGT CAT GAT TGG ATT TTT GAT GAA TTA GAG AGT TTA GGA TAT AAA CAC 432  
 Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His  
 130 135 140

30 GAA GGA TTC CAC AAA GGA TTT GAT CCT GTA TTA CAA ATC CGA TAT CAT 480  
 Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His  
 145 150 155 160

35 TCT GTT CTA AAT TTA GCA AAC AAA AGT GCT AAT GAT GTT TTA AAA AAC 528  
 Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn  
 165 170 175

ATG GAT GGT TTA AGA AAG CGT AAT ACT AAA AAA GTT AAG AAA AAT GGA 576  
 Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly  
 180 185 190

40 GTT AAA GTC CGC TTT TTA TCT GAA GAA GAG TTA CCT ATA TTT AGG TCA 624  
 Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser  
 195 200 205

45 TTT ATG GAG GAT ACC TCT GAA ACT AAA GAT TTT GCA GAT AGA GAA GAT 672  
 Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp  
 210 215 220

50 AGT TTT TAT TAC AAC AGA TTC AAA CAT TAT AAA GAC CGT GTT TTA GTA 720  
 Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val  
 225 230 235 240



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CCA CTA GCC TAT ATT AAC TTT GAT GAG TAT ATA GAG GAA CTA AAT AAT 768  
Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn  
245 250 255

5 GAA AGA AAT GTG CTT AAT AAA GAT TAT AAT AAA GCT TTA AAA GAC ATT 816  
Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile  
260 265 270

10 GAG AAA CGT CCA GAG AAT AAA AAA GCA CAT AAC AAA AAG GAA AAT TTA 864  
Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu  
275 280 285

15 GAA CAA CAA CTC GAT GCA AAT CAG CAA AAA ATT AAT GAA GCT AAA AAC 912  
Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn  
290 295 300

TTA AAA CAA GAA CAT GGC AAT GAA TTA CCC ATC TCT GCT GGC TTC TTT 960  
Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe  
305 310 315 320

20 ATA ATT AAT CCG TTT GAA GTA GTT TAC TAC GCT GGT GGA ACT TCA AAT 1008  
Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn  
325 330 335

25 CGT TAT CGC CAT TTT GCA GGG AGC TAT GCG GTT CAA TGG AAG ATG ATT 1056  
Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile  
340 345 350

AAC TAT GCA ATT GAA CAT GGT ATT AAT CCG TAT AAT TTC TAT GGT ATT 1104  
Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile  
355 360 365

30 AGT GGT GAC TTT AGT GAA GAT GCT GAA GAT GCT GGC GTA GTT AAG TTT 1152  
Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe  
370 375 380

35 AAA AAG GGC TAT GAT GCC GAT GTT ATA GAA TAC GTT GGT CAC TTT ATT 1200  
Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile  
385 390 395 400

40 AAA CCT ATT AAT AAA CCA ATG TAT AAC ATT TAT AGA ACA CTT AAA AAA 1248  
Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys  
405 410 415

CTA AAG AAA 1257  
Leu Lys Lys

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## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 419 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe
1           5           10           15

Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr
20           20           25           30

Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys
35           40           45

Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro
25           50           55           60

Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile
30           65           70           75           80

Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser
35           85           90           95

Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr
100           105           110

Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala
115           120           125

Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His
40           130           135           140

Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His
145           150           155           160

Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn
45           165           170           175

Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly
180           185           190

Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser
50           195           200           205

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	Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp	
	210	215 220
5	Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val	
	225	230 235 240
	Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn	
		245 250 255
10	Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile	
		260 265 270
	Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu	
		275 280 285
15	Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn	
		290 295 300
	Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe	
	305	310 315 320
20	Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn	
		325 330 335
	Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile	
		340 345 350
25	Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile	
		355 360 365
	Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe	
		370 375 380
30	Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile	
		385 390 395 400
	Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys	
		405 410 415
35	Leu Lys Lys	
40		
45		
50		
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGATATAAAG ATCTAGATGG GAGTTATGAA 30

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATTCATAAT TAGATGGATC CCTTCTTAAA ATC 33

**Claims**

1. A DNA compound that comprises an isolated DNA sequence encoding SEQ ID NO: 2.
2. The DNA compound of Claim 1 which comprises the isolated DNA sequence which is SEQ ID NO: 1.
3. A recombinant DNA vector that comprises the isolated DNA sequence of Claim 1.
4. A recombinant DNA vector of Claim 3 that further comprises a promoter positioned to drive expression of said isolated DNA sequence.
5. A method for constructing a recombinant host cell capable of expressing SEQ ID NO: 2, said method comprising transforming a host cell with a recombinant DNA vector that comprises an isolated DNA sequence of Claim 1.
6. A method for expressing SEQ ID NO: 2 in a recombinant host cell; said method comprising culturing said transformed host cell of Claim 5 under conditions suitable for gene expression.
7. A recombinant host cell transformed with a recombinant DNA vector of Claim 3.

8. The protein, in purified form, encoded by SEQ ID NO:2.

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FIG. 1

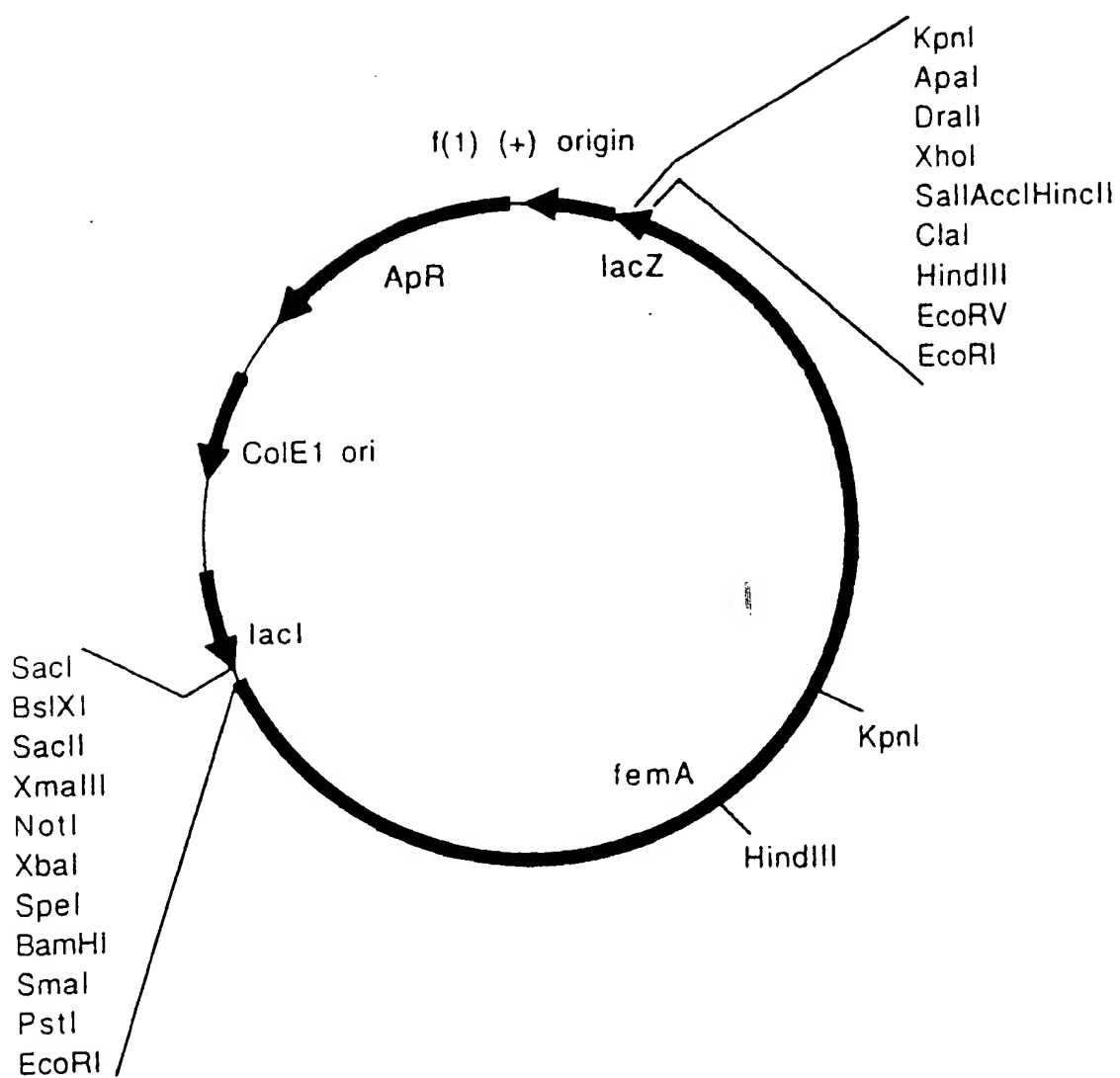
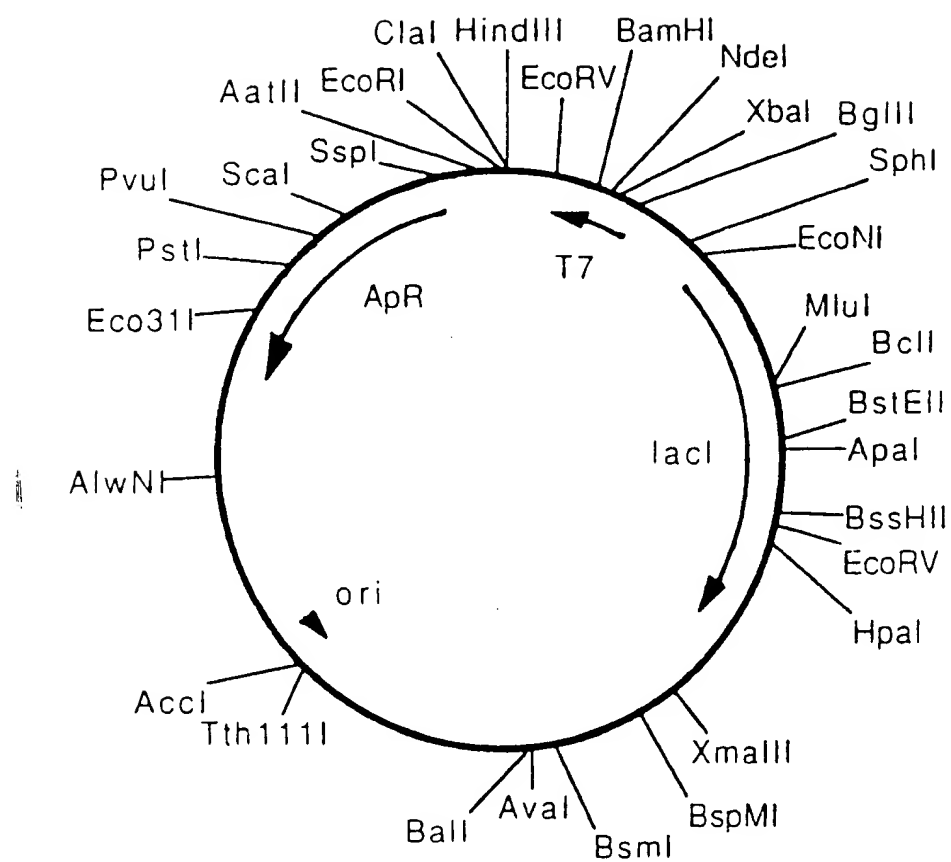


FIG. 2





Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) Publication number : **0 625 575 A3**

(12)

## EUROPEAN PATENT APPLICATION

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C07K 13/00**

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PT SE**

(88) Date of deferred publication of search report :  
**22.02.95 Bulletin 95/08**

(71) Applicant : **ELI LILLY AND COMPANY**  
**Lilly Corporate Center**  
**Indianapolis Indiana 46285 (US)**

(72) Inventor : **Alborn, William Ernest, Jr.**  
**1320 Thistlewood Court**  
**Carmel, Indiana 46032 (US)**  
Inventor : **Hoskins, Jo Ann**  
**8229 Tern Court**  
**Indianapolis, Indiana 46256 (US)**  
Inventor : **Skatrud, Paul Luther**  
**2412 Lake Crossing**  
**Greenwood, Indiana 46143 (US)**  
Inventor : **Ünal, Serhat**  
**Yesilyurt sok. No: 7/8**  
**06690 Kavakalidere, Ankara (TR)**

(74) Representative : **Hudson, Christopher Mark et al**  
**Lilly Industries Limited**  
**European Patent Operations**  
**Erl Wood Manor**  
**Windlesham Surrey GU20 6PH (GB)**  
**Declaration under Rule 28(4) EPC (expert solution)**

(54) **Fem A gene of staphylococcus epidermidis, fem A protein, and vectors of microorganisms comprising the fem A gene.**

(57) The instant invention provides the *femA* gene of *Staphylococcus epidermidis* and all degenerate sequences thereof, the protein encoded by the *femA* gene (FemA), and vectors and microorganisms comprising genes encoding the FemA protein.

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European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 94 30 2950

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
Y	MOL. GEN. GENET., vol.219, 1989 pages 263 - 269 BERGER-BÄCHI, B. ET AL. 'FemA, a host-mediated factor essential for methicillin resistance in Staphylococcus aureus: Molecular cloning and characterization' see Table 1 and Figure 4 ---	1-8	C12N15/31 C12P21/02 C07K13/00
Y	ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol.36, no.12, December 1992 pages 2617 - 2621 HÜRLIMANN-DALEL, R. ET AL. 'Survey of the methicillin resistance-associated genes mecA, mecR1-mecI, and femA-femB in clinical isolates of methicillin-resistant Staphylococcus aureus' see Figure 1 ---	1-8	
A	WO-A-91 08305 (U-GENE RESEARCH) 13 June 1991 see pages 1-4 -----	1	TECHNICAL FIELDS SEARCHED (Int.Cl.5)  C07K
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 15 December 1994	Examiner Alt, G
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	

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